Pre-baiting for increased acceptance of zinc phosphide baits by voles: an assessment technique

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Abstract: During a product-performance test of 2% zinc phosphide (Zn_3P_2) steam-rolled-oat groats (11.2 kg ha^{-1}) to control voles (*Microtus spp*) in alfalfa (*Medicago sativa*), randomly located, brushed-dirt plots were used to assess broadcast distribution and removal/acceptance of placebo particles. Results showed that the Spyker$^{®}$ Model-75 Spreaders were calibrated adequately, with placebo baits broadcast uniformly onto plots [$\bar{x} \pm SD = 3.5$ (± 2.7) groats 930 cm^{-1}]. Acceptance of the placebos by voles increased rapidly – 28% and 60% by 24 h and 48 h post-broadcast, respectively. Analyses of variance confirmed the uniformity (non-significance) of particles broadcast among enclosures/plots and the significantly greater removal/acceptance of placebos across days. This technique affords an objective decision-making tool for applicators and researchers applying Zn_3P_2 baits in field situations – an objective technique of assessing pre-bait acceptance that should improve efficacy of the rodenticide. © 1999 Society of Chemical Industry

Keywords: acute rodenticide; bait effectiveness; *Microtus spp*; pre-baits; vole control; zinc phosphide

1 INTRODUCTION

Zinc phosphide (Zn_3P_2, CAS #1314-84-7) is a widely used acute rodenticide;[1–3] however, rodent avoidance of baits due to sublethal toxicosis and learned aversion (bait shyness) can decrease acceptance and efficacy.[4,5] Mitigation of bait shyness usually involves pre-bait applications – initial distribution of placebo baits to increase acceptance of novel foods (reduced neophobia). This is viewed as increasing the rate of ingestion and thereby reducing the likelihood of sub-lethal toxicosis associated with the slow build-up of phosphine gas, as few Zn_3P_2 baits hydrolyse upon contact with the moist tissues in the rodent's gastrointestinal (GI) tract.[6–9]

Two key issues facing applicators and researchers attempting to apply Zn_3P_2 are the utility of pre-bait applications and the assessment of pre-bait effectiveness. This paper describes the use of randomly located, brushed-dirt plots to determine the broadcast distribution of pre-baits (equipment calibration) and the acceptance of pre-bait particles by target rodents. The technique offers an objective, decision-making approach to improve the timing of Zn_3P_2-bait application and thereby efficacy of the rodenticide. The Zn_3P_2 baits are applied (timed) only after sufficient removal of pre-bait particles is observed empirically.

2 MATERIALS AND METHODS

Data were collected as part of a product-performance study of Zn_3P_2 efficacy.[10–12] That study evaluated a single pre-bait and test-bait broadcast of 0% or 2% Zn_3P_2 steam-rolled-oat groats (11.2 kg ha^{-1}) to control voles (*Microtus spp*) in alfalfa (*Medicago sativa* L).[12]

2.1 Study site

The study site was the Hyslop Crop Science Field Laboratory (HCSFL) operated by Oregon State University. This agricultural facility is located c10 km north of Corvallis, Oregon. The terrain is level (elevation about 70 m), consisting of a Woodburn silty-clay soil (pH 6.0).

2.2. Enclosures

Eighteen 0.2-ha (45 × 45 m) enclosures were used. Enclosures were constructed of galvanized sheet metal; the metal extended c 1 m above ground and 0.6 m below ground. Adjacent panels were joined to form a continuous barrier to rodents. A stand of alfalfa had been planted within enclosures 2.5 years before the current study.

2.3 Voles

A total of 428 gray-tailed voles (*Microtus canicaudus*)

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were used. The voles were trapped and ear-tagged at HCSFL.

2.4 Brushed-dirt plots

Eight randomly located, 930-cm^2 (30.5 × 30.5 cm), brushed-dirt plots were prepared within each enclosure; this equated to a sampling area of 3.72 m^2 ha^{-1}. Plots were cleared of alfalfa plants/debris, covered with a thin layer of finely sieved soil, and brushed smooth with a 10.2-cm-wide commercial paint brush. Each square was marked with a small white flag. Use of random, fixed-location brushed-dirt plots affords relatively quick determinations of pre-bait removals, and typical tail-drag/footprint patterns may permit identification of the family/genus of rodents involved.

2.5 Placebo baits

Placebo baits (pre-baiting) were formulated by weight as 98.84% groats, 0.52% Alcolec-S (American Lecithin Co, Woodside, New York), 0.52% mineral oil, plus 0.12% special ingredients (confidential statement of formula).

2.6 Procedures

After a 10-night trapout to ensure that voles were not present in enclosures, 23 or 24 ear-tagged voles were distributed in each enclosure; vole densities were c 120 voles ha^{-1}. Voles were then allowed from six to 18 days for acclimation.

Calibration of Spyker^R Model-75 Spreaders (Spyker Co, N Manchester, Indiana) preceded pre-baiting of enclosures. Several manual broadcasts of placebo baits (11.2 kg ha^{-1}) by two applicators (Cenex Land O' Lakes, Tangent, OR) in nearby alfalfa comprised this calibration. Briefly, 2072-g quantities (11.2 kg ha^{-1} adjusted for 0.2 ha with a 5-m-wide, no-bait edge) of placebo groats were pre-weighed into plastic bags; next, the 'spread width' (≈2.5 m) and 'rate adjustment' screws (moderate) were set on each Spyker^R Model-75 Spreader. After emptying the pre-weighed groats into each Spyker^R hopper, the applicators were instructed to dispense half of the bait onto half of an unused 0.2-ha enclosure in adjacent, c 2.5-m-wide swaths. Remaining weight of the bait (g) was then used to compute the accuracy of the broadcast. Data showed that 46%, 35%, and 42% of the pre-weighed bait was dispensed during three successive calibration trials by the first applicator; whereas, data showed that 79% and 50% of the pre-weighed bait was broadcast during two calibration trials by the second applicator.

Upon completion of equipment calibrations, 2072-g quantities of placebo groats were broadcast in each of the 18 enclosures. Each applicator walked and manually cranked the Spyker^R spreader in alternate passes of c 2.5-m-wide, adjacent swaths over the entire enclosure.

Immediately following broadcast, each brushed-dirt plot was checked for groats. Placebo bait particles were counted and removed; subsequently, four placebo groats were placed on each plot (1 per 232.5-cm^2 quadrant of each 930-cm^2 plot). Thus, a total of 32 baits were available on the eight plots within each enclosure.

Plots were monitored for particle removals one and two days (c 0800–0900 h) after pre-baiting and placement of four pre-baits per plot. [Note. The generic procedure would call for plots to be monitored daily until the established 'pre-bait' removal criterion set by the applicator/researcher was met.] After recording the number of pre-bait particles remaining in each quadrant on day 1, the day-old groats were removed using metal tweezers. A thin layer (c 1 cm) of finely sieved soil was then reapplied to each plot and brushed smooth. Finally, four new placebo groats were placed within each plot; replacement of baits on plots allowed direct computation of each day's acceptance (percent active), and aided determination of pre-bait removals by using fresh, unsoiled particles.

2.7 Data analysis

The uniformity of pre-bait particles broadcast (equipment calibration) onto plots was assessed using a one-way analysis of variance (ANOVA; 18 enclosures).[13,14] Removal of pre-bait particles across the two days of exposure was analyzed using a two-way ANOVA (18 enclosures × 2 days), with days considered a repeated measures factor.[13,14] Significant sources of variance were analyzed using *post hoc* Newman–Keuls mean comparisons,[13,14] all statistical tests involved the 0.05 alpha level. Acceptance was computed both as the percentage of particles removed daily relative to those available [(particles absent ÷ 576) × 100] and the percentage of plots having ≥1 groat(s) missing relative to total plots [(# plots with ≥1 groat(s) missing ÷ 144) × 100].

3 RESULTS

3.1 Broadcast uniformity

The ANOVA for particle distribution yielded no significant effect among enclosures for placebo bait particles found on plots post-broadcast ($F = 1.39$; 17, 126 df; $P > 0.15$). Although this result would suggest uniformity of pre-bait distributions among enclosures, sizable variances characterized the distributions of pre-baits on plots among enclosures (Table 1). A mean (±SD) of 3.5 (±2.7) groats were broadcast onto the 144 plots during pre-baiting, with a minimum–maximum groats per plot of 0–17. Within enclosures (8 plots), mean (±SD) bait particle distributions varied between 1.75 (±0.88) and 5.25 (±2.96) groats (Table 1).

3.2 Pre-bait acceptance

Table 2 presents the pre-bait removal data for days 1 and 2. The ANOVA for particles removed from plots yielded significant interaction and main effect terms: enclosure × day ($F = 3.67$; 17, 126 df; $P < 0.0001$), enclosure ($F = 3.72$; 17, 126 df; $P < 0.0001$), and day

Enclosure	Plot								Mean (±SD)
	1	2	3	4	5	6	7	8	
1	7	5	2	2	5	3	2	1	3.37 (±2.06)
2	2	0	8	1	3	6	2	6	3.50 (±2.82)
3	4	1	2	2	3	0	2	3	2.12 (±1.24)
4	8	4	7	4	2	3	5	8	5.12 (±2.29)
5	2	2	2	3	2	1	0	2	1.75 (±0.88)
6	3	1	8	3	1	6	1	3	3.25 (±2.55)
7	4	2	2	8	1	5	2	1	3.12 (±2.41)
8	2	6	3	6	3	5	4	6	4.38 (±1.60)
9	9	0	4	4	4	5	8	8	5.25 (±2.96)
10	12	4	3	3	3	4	5	5	4.88 (±3.00)
11	1	0	5	5	6	1	6	3	3.37 (±2.44)
12	1	4	4	4	1	4	12	2	4.00 (±3.50)
13	0	0	2	1	2	6	1	5	2.12 (±2.23)
14	5	1	3	8	0	3	4	3	3.37 (±2.44)
15	3	3	1	4	1	2	3	5	2.62 (±1.41)
16	4	1	0	3	5	0	3	6	2.75 (±2.25)
17	0	17	6	4	3	3	0	8	5.12 (±5.51)
18	1	3	1	2	2	4	5	5	2.87 (±1.64)

Table 1. Distributions of steam-rolled-oat groats found within plots immediately after the pre-bait broadcasts.

($F = 121.92$; 1, 126 df; $P < 0.0001$). A complex pattern of mean particle removals characterized the enclosure × day interaction; essentially, these reflected the increased acceptance of particles between days 1 and 2. *Post hoc* Newman–Keuls mean comparisons revealed that mean particle removal was greater for day 2 than for day 1, but specific enclosure × day cells differed more than others. Enclosure 10 was an outlier, yielding means (±SD) of 0.12 (±0.35) and 4.00 (±0.00) particle removals per plot across days, whereas particle removals for enclosure 2 were more equivalent for both days, yielding mean (±SD) removals of 1.50 (±1.85) and 1.75 (±1.58) for days 1 and 2, respectively. This mix of different mean particle removals among cells of the design, coupled with a general increase for day 2, accounted for the interaction.

Regarding the main effects, *post hoc* Newman–Keuls tests clearly showed that the enclosure effect was due to the differences among particle removals for certain enclosures. For example, mean (±SD) removals for enclosures 1, 3, and 17 (combined across days) were 0.94 (±1.12), 0.56 (±1.03), and 0.50 (±0.82), whereas mean (±SD) removals for enclosures 5, 6, 10, 12, 13, 14, and 18 were 2.31 (±1.49), 2.87 (±1.54), 2.06 (±2.02), 2.25 (±1.34), 2.94 (±1.44), 2.94 (±1.43), and 2.87 (±1.63), respectively – removals ranging from <1 particle to >2 particles per plot. Finally, mean (±SD) particle removals were 1.19 (±1.47) and 2.45 (±1.59) for days 1 and 2, respectively – evidence of increased 'acceptance'.

Numbers of groats removed daily proved to be a more conservative index of acceptance than the plot counts with ≥1 pre-baits missing. This is probably a numerical artefact of the counts. Removal of a single pre-bait groat from a plot leads to 0.0069% increase in plot counts for the 144 plots, but this only adds

0.0017% to the maximum 576 pre-baits placed on plots. Of the 576 particles placed on plots daily, 166 and 348 placebo baits were missing after one and two days, respectively – 29% and 60% acceptance. Regarding the numbers of plots having ≥1 pre-bait(s) removed, 70 and 118 of the 144 plots met this criterion after one and two days, respectively – 49% and 82% acceptance, respectively.

4 DISCUSSION

The technique confirmed that pre-baits were broadcast uniformly, with empirical counts of particles on plots confirming the calibration of Spyker[R] spreaders. Broadcast of 2.5 to 5 groat particles onto 930.25-cm^2 plots is considered typical for a 11.2 kg ha^{-1} (10 1b acre^{-1}) broadcast (Salmon, pers comm, 1993). Mean particle distributions in 14 of the 18 (78%) enclosures were in this range, with one outlier accounting for practically all of the observed pre-baiting dispersion – Enclosure 17 [x̄ (±SD) = 5.1(±5.5); range = 17]. Although the clearing of the plant overgrowth to prepare brushed-dirt plots could be viewed as a source of bias affecting bait distribution onto plots, mean (±SD) alfalfa height in enclosures was >28 (±14) cm. Direct broadcast of pre-baits onto plots was atypical; deflections of baits from adjacent alfalfa plants characterized the applications. Thus, count distributions of particles broadcast onto plots was considered typical of actual alfalfa applications.

The pattern of pre-bait removals from some plots and not others between days 1 and 2 supports the pre-baiting approach. Voles in 16 of 18 enclosures foraged more groats from a greater number of plots on the second day. This suggests that voles either gradually expanded their foraging areas to include other plots or

		Plot									
Enclosure	Day	1	2	3	4	5	6	7	8	Σ	Plots with ≥1 removal
1	1	1	0	0	2	0	2	0	0	5	3
	2	1	1	0	3	0	2	3	0	10	5
2	1	0	0	0	4	1	0	3	4	12	4
	2	0	1	2	4	1	0	2	4	14	6[a]
3	1	0	0	0	0	1	0	0	0	1	1
	2	1	0	0	1	1	4	1	0	8	5
4	1	4	0	1	4	2	0	4	0	15	5
	2	4	0	1	4	3	0	4	0	16	5
5	1	2	0	4	0	3	3	2	1	15	6[a]
	2	3	0	4	1	3	4	4	3	17	7[a]
6	1	4	4	0	0	1	3	1	2	14	6[a]
	2	4	4	4	4	4	4	3	4	31	8[a]
7	1	1	2	4	0	0	1	1	4	13	6[a]
	2	4	4	3	0	0	1	2	4	18	6[a]
8	1	0	0	0	0	2	0	0	4	6	2
	2	0	0	2	0	4	4	4	4	18	5
9	1	1	0	1	0	1	0	0	0	3	3
	2	2	0	4	2	4	2	3	1	18	7[a]
10	1	0	1	0	0	0	0	0	0	1	1
	2	4	4	4	4	4	4	4	4	32	8[a]
11	1	0	0	0	1	0	0	0	3	4	2
	2	4	4	1	0	4	0	4	1	18	6[a]
12	1	1	0	4	2	4	2	3	0	16	6[a]
	2	1	2	1	4	3	4	2	3	20	8[a]
13	1	3	1	3	2	0	1	1	4	15	7[a]
	2	4	4	4	4	4	4	4	4	32	8[a]
14	1	4	3	4	3	0	4	2	0	20	6[a]
	2	4	3	4	3	4	4	4	1	27	8[a]
15	1	3	2	1	1	0	0	1	0	8	5
	2	4	2	1	1	0	3	2	0	13	6[a]
16	1	1	0	0	0	0	0	3	0	4	2
	2	4	2	4	0	1	1	3	1	16	7[a]
17	1	0	0	0	0	0	0	0	0	0	0
	2	1	0	2	2	2	0	1	0	8	5
18	1	0	0	3	4	3	0	1	3	14	5
	2	4	4	4	4	4	4	4	4	32	8[a]

Table 2. Numbers of removed steam-rolled-oat groats and number of plots with ≥1:4 groats missing daily.

[a] Indicates that criterion of ≥1 groat missing from ≥6 plots was met.

that voles with core ranges in certain parts of the 0.2-ha enclosures were initially neophobic of the groats.

The particle-removal data allowed calculation of diverse acceptance indices. Nevertheless, detections/computations of percentage missing particles was more time-consuming than those of whether 1 or more particles were removed from plots. Use of 'plot-acceptance' criteria will speed rodenticide bait decisions in most field situations. The absence of ≥1:4 (≥25%) groats from ≥6:8 (≥75%) plots within ≥9:18 (≥50%) enclosures was an arbitrary criterion used to determine the day of Zn_3P_2-bait broadcast in the product-performance study.[12] A criterion of greater or lesser stringency could be used by applicators/researchers to adjust the timing of Zn_3P_2-bait applications. Still, numerous 'real-world' factors ultimately affect the timing and effectiveness of rodenticide use – acceptance is only one of these. For example, the likelihood of precipitation is critical with Zn_3P_2, due to

hydrolysis of the rodenticide and deterioration of grains with lecithin adhesives under moist conditions, and the locations/densities of voles within fields should ultimately determine areas of rodenticide application.

5 CONCLUSIONS

Use of multiple, 930-cm^2, brushed-dirt plots containing fixed numbers of placebo bait particles afforded an empirical basis for timing Zn_3P_2-bait broadcast. Rodenticide application was set to occur whenever the criterion of '≥one pre-bait groat was removed from 75% of plots in 50% of enclosures'; this empirical pre-baiting criterion undoubtedly contributed to the 94.6% efficacy achieved in the cited product-performance study.[12] Although two successive days of pre-bait exposure were appropriate in this study, length of exposures will vary. Neophobia, seasonality, bait formulation, rodent density, and rainfall are key

factors that will determine the length of pre-bait exposure and the number of pre-bait applications needed to attain the acceptance criterion.

REFERENCES

1 Merson MH and Byers RE, *Crop Prot* **4**:511–519 (1985).
2 Sterner RT, *Proc Vertebr Pest Conf* **16**:152–159 (1994).
3 Tietjen HP, Zinc phosphide –its development as a control agent for black-tailed prairie dogs. Special Sci Rept (Wildl No 195), US. Department of the Interior, Washington, DC, 14 pp (1976).
4 Gratz NG, *Bull Wld Hlth Org* **48**:469–477 (1973).
5 Marsh RE, Forest Service General Technical Report (RM-154), United States Department of Agriculture, Washington, DC, pp. 70–74.
6 Murphy SD, In *Casseratt and Doull's Toxicology: The Basic Science of Poisons* (3rd edn), ed by Klaussen CD Amdur MO and Doull J, Macmillan, New York, pp 566–567 (1986).
7 Sterner RT and Mauldin RE, *Arch Environ Contam Toxicol* **28**:519–523 (1995).
8 Tietjen HP and Matschke GH, *J Wildl Manage* **46**:1108–1112 (1982).
9 Tkadlec E and Rychnovsky B, *Folia Zool*, **39**:147–156 (1990).
10 US Environmental Protection Agency, In *Pesticide Assessment Guidelines, Subdivision G, No 540/9-82-026*, ed by Schneider BA and Hitch RK, Office of Pesticide Programs, Washington DC, pp 1–49, 313–315, 337–39 (1982).
11 US Environmental Protection Agency, *Code of Federal Regulations: Protection of Environment 40* (Parts 150–89), US Government Printing Office, Washington DC, 757 pp (1993).
12 Sterner RT, Ramey CA, Edge WD, Manning T, Wolff JO and Fagerstone KA, *Crop Prot* **15**:727–734 (1996).
13 Winer BJ, *Statistical Principles in Experimental Design*, McGraw-Hill, New York, pp 196–218, 309–335 (1971).
14 SAS Institute, *SAS/STAT Guide for Personal Computers*. SAS Institute, Inc, Cary, North Carolina, pp 125–154 (1987).